Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/CA04/002202

International filing date: 24 December 2004 (24.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/534,346
Filing date: 06 January 2004 (06.01.2004)

Date of receipt at the International Bureau: 23 March 2005 (23.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

PCT/CA 2004/002202

09 FEBRUARY 2005 09.02.05

CA04/02202

PA 1266469

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 03, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/534,346***
FILING DATE: ***January 06, 2004***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

H. L. JACKSON
Certifying Officer

Please type a plus sign (+) inside this box → [+]

PTO/SB/16 (5-03)
Approved for use through 4/30/2003 OMB 0651-0032
U S Patent and Trademark Office, U S DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)		
Given Name (first and middle (if any))	Family Name or Surname	Residence (City and either State or Foreign Country)
Jean-Guy	LEHOUX	Sherbrooke, Quebec, CANADA
Gilles	DUPUIS	Sherbrooke, Quebec, CANADA

☐ *Additional inventors are being named on the ____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

A Simplified Method to Retrieve Chitosan From Acidic Solutions Thereof

Direct all correspondence to. **CORRESPONDENCE ADDRESS**

☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*

OR *Type Customer Number here*

☒ Firm or Individual Name: Julie GAUVREAU, Goudreau Gage Dubuc

Address	Stock Exchange Tower				
Address	800 Place-Victoria, Suite 3400				
City	Montreal	State	Quebec	ZIP	H4Z 1E9
Country	CANADA	Telephone	(514) 397-4374	Fax	(514) 397-4382

ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification *Number of Pages* 16 ☐ CD(s), Number
☐ Drawing(s) *Number of Sheets* ☐ Other (specify)
☐ Application Data Sheet See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☒ Applicant claims small entity status See 37 CFR 1.27
☐ A check or money order is enclosed to cover the filing fees
☒ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 07-1742
☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.
☐ Yes, the name of the U S. Government agency and the Government contract number are _____

Respectfully submitted.

SIGNATURE [signature]

TYPED or PRINTED NAME Julie GAUVREAU

TELEPHONE (514) 397-4374

Date

REGISTRATION NO. (if appropriate) 52,532

Docket Number. 10857.367

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTMALUREV05

Our file: 10857.367
Ser. No.: Unassigned
Filed: Concurrently herewith
Applicants: LEHOUX, Jean-Guy and DUPUIS, Gilles

Enclosures:

- English Text (16 pages); and
- Provisional Application Cover Sheet.

PLEASE STAMP DATE AND RETURN

TITLE OF THE INVENTION

[0001] A SIMPLIFIED METHOD TO RETRIEVE CHITOSAN FROM ACIDIC SOLUTIONS THEREOF

FIELD OF THE INVENTION

[0002] The present invention relates to a simplified method for retrieving chitosan from aqueous acidic solutions. More specifically, the present invention concerns a method for retrieving chitosan from aqueous acidic solutions by addition of chaotropic salts.

BACKGROUND OF THE INVENTION

[0003] Chitosan is the deacetylated form of chitin, which is a linear polymer of acetylamino-*D*-glucose and contains high contents of amino and hydroxyl functional groups. This polycationic polymer is usually prepared commercially by limited hydrolysis of naturally occurring chitin from the exoskeleton of crustaceans and insects. Chitin is a polymer composed of *N*-acetyl-β-*D*-glucosamine (2-acetamido-2-deoxy-β-*D*-glucopyranose) monomeric units whereas commercially available chitosan is a heterogenous mixture of molecular weight and sizes of chitin deacetylated to various extents.

[0004] Chitosan possesses a wide variety of commercial and biomedical applications that are related to the size of the molecule and its degree of acetylation. With respect to biomedical applications, it has been reported that the hypocholesterolemic efficiency of chitosan increases in an inverse relationship to its size and percentage of acetylation (1,2). Other studies have reported that chitosan molecules of 25 to 50 kiloDaltons (kDa) are efficient in the treatment of stomach ulcers (3) whereas chitosan molecules of 28 kDa have been used as nanoparticles for the controlled release of drugs (medications) (4). Low molecular weight chitosans (LMWC) (2 kDa) have been used in agriculture as anti-fungal agents to protect tubercules, salad and tobacco seeds (5). In contrast, chitosan of 400 kDa has been shown to be a suitable vehicle in a DNA vaccination approach of desensitization to

peanut allergens in mice (6). These few examples illustrate the remarkable array of applications of chitosan and the importance of the size of the chitosan molecule for specific applications. It follows that targeted applications of chitosan require a well-characterized product that must be prepared under rigorously r producible conditions.

[0005] The molecular sizes of commercially available chitosans generally vary between 70 kDa and more than 1000 kDa, whereas the percentage of deacetylation is usually in the range of 50-100%. The percentage of deacetylation of chitin and its depolymerization to yield chitosan are a function of the conditions of the chemical treatment with aqueous base. Extended treatments lead to more fragmented molecules of chitosan, a property known as polydispersion. Polydisperse chitosan preparations are less desirable based on the observations of its size- and deacetylation-related properties, as discussed above.

[0006] Controlled enzymatic hydrolysis of commercially available chitosan polymers is the only reproducible method that exists to generate a product possessing a low dispersity and defined molecular weight properties. The physical characteristics of the starting material (chitosan) are important (size, percentage of acetylation) because they will influence the conditions of enzymatic digestion. Commercial chitosans vary in size and this property influences the time required for the production of depolymerized chitosans of defined molecular sizes for commercial applications in the areas of biomedicine, agriculture, cosmetics and others.

[0007] Enzymatic digestion of chitosan with a chitosanase is the only method that can be used to reproducibly generate LMWC with a low degree of polydispersity. Chitosanase is an enzyme that possesses a high degree of specificity for chitosan (7). Chitosan digestion with chitosanase is performed in a weakly acidic solution. Several experimental conditions must be controlled, among which are:

[0008] The enzymatic digestion must be rapidly stopped to prevent further depolymerization of chitosan and the generation of a polydispersed product.

[0009] An easy-to-use methodology must be employed to isolate the product of digestion rapidly and in a solid form that facilitates drying, ideally into a powder form. This consideration is highly desirable when large quantities (e.g. several hundred kilograms) of chitosan are to be processed for commercial purposes.

[0010] The product of digestion must be free of the enzyme (chitosanase).

[0011] The hydrolyzed product must be isolated under conditions that make it fit for human uses, especially when applications to the biomedical field are sought.

[0012] In the case of the use of chitosan as a food supplement, the product of hydrolysis must easily dissolve in an aqueous acidic milieu such as the one of the stomach. This condition limits the number of methodologies that can be used to retrieve the product from the acidic solution used for its enzymatic digestion or from other processes.

[0013] A number of methodologies have been used to isolate chitosan from aqueous acidic solutions. The most commonly used technique is to decrease the solubility of chitosan by raising the pH through addition of an inorganic base (as an example, sodium or potassium hydroxide). This procedure is very efficient to precipitate chitosan from such solutions but it suffers from the fact that the resulting mixture is highly viscous, making the isolation of precipitated chitosan difficult by conventional techniques of separation. Furthermore, the desired product must be free of excess base. This can be accomplished only at the expense of extensive washings, an approach that is time-consuming and that results in an appreciable loss (mechanical or by dissolution) of chitosan. Another aspect that ought to be taken into account when chitosan is hydrolyzed by treatment with a chitosanase is the possibility that chitosanase is concomitantly precipitated and may still remain active due to its robustness to pH treatment and/or remains as a contaminant in the processed product after the precipitate has been freed of excess base. One further point of paramount importance is that the final product must be free of contaminating

alkali, esp cially if it is to be used for biomedical purposes.

[0014] An often efficient method of precipitation of chitosan from aqueous solutions consists in the addition of polyphosphoric acid (8) or polyphosphate salts (9). The phosphate salts of chitosan are insoluble in aqueous media. However, the major drawback of this method is that the phosphate salts of chitosan are poorly soluble in a physiological acidic environment such as the gastric milieu of the stomach.

[0015] It is of interest to point out further, that heat has been used to stop the action of chitosanase when chitosan is prepared by treatment with a chitosanase. In this instance, the inherent stability of chitosanase to heat denaturation requires raising the temperature of the reaction to 60°C or more. This temperature favors the well-described and known Maillard reaction which leads to partial decomposition of chitosan and the generation of colored products resulting from the reaction of the primary amines of the chitosan molecules. This behavior is highly undesirable since these same amino groups are important for the biological properties of chitosan. Two additional points are of further interest. First, heat-denatured chitosanase may precipitate and be carried over in the subsequent steps of isolation of chitosan (e.g. by precipitation). Second, the partial resistance of chitosanase to heat denaturation may allow its renaturation and partial recovery of activity, adding to the possibility of further digestion of chitosan.

[0016] Overall, the current methods of precipitation and isolation of chitosan from chemical or enzymatic hydrolysates are therefore not adequate. Easy-to-use methodologies enabling high yields of a product suitable for commercial uses, and especially of biomedical uses are of the utmost interest. The current art to isolate chitosan from aqueous acidic solutions, by raising the pH thereof by the addition of alkali or the formation of insoluble salts of chitosan do not fulfill these requirements.

[0017] Therefore, there remains a need to provide a simple, reliable, reproducible method for retrieving chitosan from chemical or enzymatic hydrolysates.

[0018] There is also a need for a method for retrieving chitosan from acidic solutions which would give a product free of contaminants (e.g. chitosanase, undesirable salts).

[0019] More particularly, there is a need for a method for retrieving high yield of chitosan suitable for commercial uses, and especially for applications related to the food and biomedical industries.

[0020] In addition, there is a need for a method for retrieving chitosan from acidic solutions which would yield chitosan which is easily dissolved in an aqueous acidic milieu that is compatible with human use.

[0021] The present invention seeks at satisfying these and other needs.

SUMMARY OF THE INVENTION

[0022] The present invention therefore broadly provides a chitosan preparation and method of preparation thereof which overcome the defects of the preparations and methods of the prior art.

[0023] In one embodiment, the present invention concerns a method for retrieving chitosan from aqueous acidic solutions. More specifically, the present invention relates to a method for retrieving chitosan from aqueous acidic solutions by the addition of chaotropic salts, and preferably food compatible and biomedically compatible inorganic or organic salts.

[0024] It is one object of the present invention to provide a method for retrieving chitosan from acidic solution by means of the addition of a chaotropic agent such as the salt of an inorganic acid or, in one particular embodiment, the salt of an organic acid suitable for human ingestion. The addition of such salt creates a salting out effect by reorganizing water molecules with the added salt, resulting in the dehydration of the dissolved chitosan molecules and their precipitation. In one embodiment, the salting out reaction is performed under non-denaturating

conditions. In one particular embodiment, the non-denaturing conditions comprise non-limiting pH values which are between about 3 and about 7 and, in another embodiment the non-limiting temperature values are between about 4°C and about 55°C. The terminology "about" used herein is meant to designate a variation of about 10%. In yet another embodiment the pH values are between 3 and 7 and the temperature between 4°C and 55°C

[0025] The present invention further relates to a method for retrieving chitosan from acidic solutions, such retrieved chitosan having conserved the physical properties of native chitosan such as ionic charges and molecular sizes. The foregoing invention also relates to a preparation of such chitosan.

[0026] In addition, the present invention relates to a method for precipitating chitosan from acidic solutions enabling the production of a precipitated chitosan preparation having a non-viscous, fiber-like appearance. Therefore, such a chitosan preparation can easily be recovered from acidic solutions using simple conventional techniques such as ultrafiltration, centrifugation, or other known and usual methods of recovery of a solid phase from a liquid phase.

[0027] Further, the invention relates to a method of retrieving chitosan from acidic solutions which is suitable for purification from enzymatic hydrolysates. The method of the present invention enables the selective precipitation of chitosan over chitosanase. This selective precipitation prevents further hydrolysis of chitosan thus reducing its polydispersity and yielding, in one embodiment, a chitosan preparation which is substantially free of chitosananse

[0028] The present invention thus also relates to a chitosan preparation, which contains negligible amounts of chitosanase. The present invention provides a method to recover chitosan from acidic solutions, such recovered chitosan preparation can be easily freed of the precipitating salt as well as other soluble substances. The chitosan preparations of the present invention achieve recovery levels of at least 90%, preferably at least 95% and most preferably at least 98%.

[0029] In addition, the invention relates to a method for purifying chitosan from acidic solutions and to a preparation of chitosan obtained therefrom. Such purified chitosan preparation is suitable for human or animal consumption and therefore satisfies the criteria required in biomedical applications or as a food additive.

[0030] In another embodiment, the invention relates to a method for purifying chitosan of various molecular sizes. The chitosan precipitate obtained by the methods of the present invention can easily be dried, and the ensuing powder is readily soluble in dilute organic and preferably inorganic acids such as hydrochloric acid solutions similar to the acid content of the stomach. This property is highly suitable in cases wherein the chitosan preparation is used as a food additive. The present invention therefore also relates to chitosan preparations of a chosen molecular size or sizes, which are readily soluble in dilute hydrochloric acid solutions. In a particular embodiment this dilute hydrochloric acid solution is the acid content of the stomach.

[0031] In summary, based on the disclosure herein, those skilled in the art can purify chitosan from aqueous acidic solutions by adding chaotropic salts. Chitosan purified by means of the present invention lack any modifications of the physical properties of the chitosan polymer such as residual ionic charges and molecular sizes (i.e. they retain the physiological properties of native chitosan). The methodology described here is simple, cost-cutting, easy to use and far-reaching. It allows a quick, efficient and quantitative recovery of chitosan from acidic aqueous solutions with a minimum of easy-to-perform operations while preserving the integrity of the product. The methodology can be applied without limitations with respect to the amount of dissolved chitosan that needs to be processed allowing the method of the present invention to be developped for small scale, large scale or ultra high scale preparation for commercial production. Furthermore, chitosan preparations purified by means of the present invention are suitable for human or animal consumption when a food compatible chaotropic salt is used to precipitate chitosan. This is of considerable importance in cases of application related to administration of chitosan to humans and animals such as applications related to the biomedical and food

industries.

[0032] Other advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of illustrative embodiments thereof, given by way of example only.

[0033] Unless defined otherwise, the scientific and technical terms and nomenclature used herein have the same meaning as commonly understood by a person of ordinary skill to which this invention pertains.

[0034] The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

[0035] The present invention is further illustrated by the following specific examples. The examples are provided for illustration only and should not be construed as limiting the scope of the invention in any way.

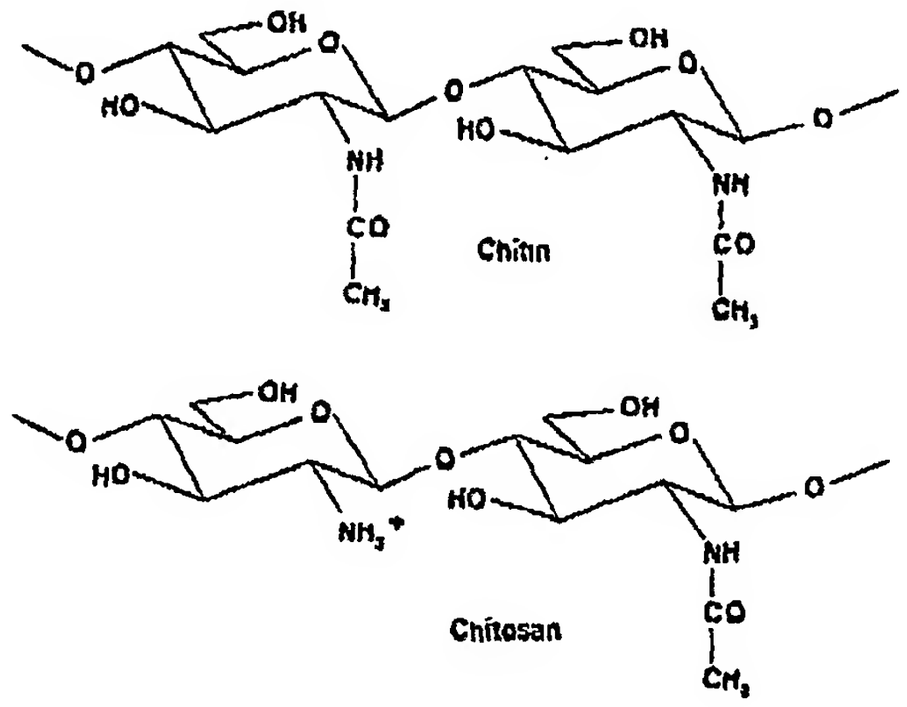
DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0036] An easy-to-use and reproducible protocol is described herein to allow high yields and quick recovery of chitosan dissolved in aqueous acidic solutions. The protocol is based on the principle of reorganization of the hydrated shell of the chitosan polymer by addition of chaotropic salts. Non-limiting examples of chaotropic salts include the sodium or potassium salts of citric acid, tartaric acid or phosphoric acid The ammonium salt of sulfuric acid can also be used effectively.

[0037] The protocol differs from previously used methods of recovery of chitosan from acidic solutions which use high pH or processes of coagulation. The method of the present invention can offer a number of advantages such as : a) safety of operation due to the use of non-corrosive reagents; b) high yields of recovery of chitosan; and c) a lack of modification of the physical properties of the chitosan polymer such as residual ionic charges and molecular sizes. The protocol is applicable to a wide range of molecular sizes of chitosan. In addition, in one

embodiment, the chitosan preparations of the present invention have the advantage of having increased stability in view of the fact that they are substantially free of chitosanase.

[0001] Chitosan is a polycationic polymer that is usually prepared commercially by limited basic hydrolysis of naturally occurring chitin, such as the exoskeleton of crustaceans and insects. Chitin is a polymer composed of *N*-acetyl-β-*D*-glucosamine (2-acetamido-2-deoxy-β-*D*-glucopyranose) monomeric units, whereas commercially available chitosan is usually composed of a heterogenous mixture of molecular sizes of chitin deacetylated to various extents. The basic schematic structures of chitin and chitosan are shown below.



Basic structures of chitin and chitosan

[0039] The present invention therefore broadly provides a chitosan and method of preparation thereof which overcome the defects of the preparations and methods of the prior art.

[0040] In one embodiment, the present invention concerns a method for retrieving chitosan from aqueous acidic solutions. More specifically, the object of the present invention is to provide a method for retrieving chitosan from aqueous acidic solutions by the addition of chaotropic salts, and preferably food compatible inorganic or organic salts.

[0041] Chitosan has a polyelectrolyte nature. Its solubility in aqueous media should thus follow rules that are similar to the mpiric rules that apply to the solubility of proteins in aqueous media. These factors are pH, temperature and ionic strength of the dissolving medium. The innovative protocol described herein is based on the sensitivity of chitosan to the *salting out* effect caused by the addition of selected electrolytes of the Hofmeister series or food-compatible organic salts. The *salting out* effect decreases the solubility of the solute by increasing the organization of water molecules around the ions instead of the solute. This salting out effect results in the *dehydration* of the solute and its precipitation.

Increasing precipitation (*salting out*) effect
Anions: $PO_4^{3-} > SO_4^{2-} >$ acetate$^- > Cl^- > Br^- > NO^{3-} > ClO_4^- > I^- > SCN^-$
Cations: $NH_4^+ > Rb^+ > K^+ > Na^+ > Cs^+ > Li^+ > Mg^{2+} > Ca^{2+} > Ba^{2+}$

Some examples of the Hofmeister series of anions and cations

[0042] The method of the present invention comprises the addition of a chaotropic salt of the Hofmeister series, preferably, a food compatible salt or a food-compatible electrolyte to an aqueous acidic solution of chitosan. Examples are given of chitosan dissolved in dilute aqueous acid (e.g. acetic acid) to which is added a chaotropic salt such as one of the followings,

- Sodium sulfate
- Sodium or potassium phosphate
- Potassium citrate
- Sodium tartrate

Of course, other dilute aqueous acidic solutions could be used.

[0043] The precipitated chitosan may be easily recovered from aqueous acid salt solution by any means known in the art including filtration, centrifigation,

evaporation, spray drying or a combination thereof.

[0044] For the purpose of this invention, a specific chitosan polymer is considered precipitated in a particular aqueous acidic solution if the said sp cific chitosan does not dissolve to form a clear homogeneous solution when the chitosan polymer is stirred or agitated for long period of time (e.g. a week) in the aqueous salt solution at a particular temperature.

[0045] A specific chitosan polymer is considered precipitated when a solution containing said chitosan in a particular aqueous acidic salt solution develops cloudiness or turbidity which it retains when the temperature of the solution is changed.

[0046] It should be understood from the foregoing that the solubility of a specific chitosan polymer in a particular aqueous acidic salt solution may be temperature dependent so that chitosan may be precipitated in an aqueous solution at lower temperature but is soluble at higher temperature or vice-versa. Therefore one can take advantage of this particularity to retrieve chitosan from particular aqueous acidic salt solution.

[0047] Chitosan may be considered to be precipitated if all or if only part of the chitosan is precipitated. Chitosan may be considered to be precipitated if at least 90%, preferably at least 95%, and more preferably at least 98% is precipitated.

[0048] The salts used in the present invention may be any chaotropic inorganic or organic salts including for example sulphates, phosphates, citrates, acetates, tartrates, fluorides and hydrogen phosphates. The counterion has a small effect and may be ammonium or any alkali or alkaline earth metal such as sodium, magnesium, calcium, potassium, lithium etc. Mixture of inorganic or organic salts are also useful.

EXAMPLE 1

CHITOSAN PRECIPITATION WITH Na_2SO_4

[0049] Twenty grams of chitosan 70 kDa obtained from Fluka (Sigma-Aldrich, St Louis, Missouri, USA) is dissolved in 5% acetic acid (500 ml). The solution is diluted with 1 liter of water and 75 g of Na_2SO_4 (final concentration, 0.35 M) are slowly added. The precipitate is kept at 4°C for 1 h and then centrifuged (8000 x g) for 20 min. The supernatant (pH 4.0) does not contain any precipitable chitosan as assayed by the addition of ammonium sulfate or polyphosphoric acid or by colorimetric assay according to the method published by Muzzarelli (10). The precipitate is washed with water (2 liters) and collected by centrifugation.

EXAMPLE 2

CHITOSAN PRECIPITATION WITH SODIUM CITRATE

[0050] Twenty grams of chitosan 70 kDa obtained from Fluka (Sigma-Aldrich, St Louis, Missouri, USA) is dissolved in 5% acetic acid (500 ml). The solution is diluted with 1 liter of water and 150 g of trisodium citrate (final concentration, 0.34 M) are slowly added. The precipitate is kept at 4°C for 1 h and then centrifuged (8000 x g) for 20 min. The supernatant does not contain any precipitable chitosan as assayed by the addition of ammonium sulfate or polyphosphoric acid. The precipitate is washed with water (2 liters) and collected by centrifugation.

[0051] Chitosan can also be retrieved from dilute aqueous acetic acid solutions by the addition of other salts such as K_2HPO_4 (0.63 M), Na/K tartrate (1.4 M) or ammonium sulfate (10 – 20% saturation).

[0052] While the invention has been described with reference to certain illustrative embodiments, those skilled in the art will appreciate that various modifications, changes, omissions and substitutions can be made without departing from the spirit and nature of the invention. For example, the effective amount of chaotropic salts or organic salts required to cause precipitation of a specific chitosan

depends on a number of factors including th concentration of chitosan in th aqueous acidic solution, the temperature, the inorganic or organic salt used, the mol cular weight of the specific chitosan, its degree of acetylation, the pH of the solution and the ambient pressure. It is understood, therefore, that the invention is not limited to the particular embodiments disclosed, but is intended to cover modifications within the spirit and scope of the present invention as defined by the appended claims.

REFERENCES

1. LeHoux, J.G. and Grondin, F. (1993) Some effect of chitosan on liver function in the rat. *Endocrinology* **132**: 1078-1084.

2. Sugano, M., Yoshida, K., Enomato, K. and Hirano, S.(1992) Hypocholesterolemic activity of partially hydrolyzed chitosans in rats. In *Advanced in Chitin and Chitosan*, (C.J. Brine, P.A. Sanford, and J.P. Zikakis, editors.) Elsevier, London, 1992: 472-478.

3. Ito, M., Ban, A. and Ishihara, M. (2000) Anti-ulcer effects of chitin and chitosan, healthy foods, in rats. *Japanese Journal of Pharmacology* **82**: 218-225.

4. Chen, X.G., Lee, C.M. and Park, H.J. (2003) Emulsification for the self-aggregation and nanoparticle formation of linoleic acid-modified chitosan in the aqueous system. *Journal of Agriculture and Food Chemistry* **51**: 3135 – 3139.

5. Beaulieu, C., Lacasse, S. and Leclerc, C. (2003) Potential use of chitosan in agriculture: growth stimulation and biological control of plants disease. *9th International Chitin-Chitosan Conference*. Montreal, Quebec, Canada, August 27-30, 2003.

6. Roy, K., Mao, H.Q., Huang, S.K. and Leong, K.W. (1999) Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nature Medicine* **5**: 387-391.

7. Brzezinski, R. (1996) Enzyme of use in chitosan hydrolysis. US patent no. 5,482,843.

8. Shu, X.Z., and Zhu, K.J. (2002) The influence of multivalent phosphate structure on the properties of ionically cross-linked chitosan films for controlled drug release. *European Journal of Pharmacology and Biopharmacology* **54**: 235-243.

9. Chiou, M.S. and Li, H.-Y. (2003) Method of adsorbing dye in aqueous solution by chemical cross-linked chitosan beads. United States Patent Application no. 20,030,101,521.

10. Muzzarelli, R.A. (1998) Colorimetric determination of chitosan. *Analytical Biochemistry* **260**: 255-257.

WHAT IS CLAIMED IS:

1. A method for precipitating chitosan compnsing: mixing in any order an aqueous acidic solution containing a chitosan polymer and at least one chaotropic inorganic or organic salt wherein said inorganic or organic salt is present in an amount effective to precipitate said chitosan polymer to form an aqueous composition which comprises at least said precipitated chitosan polymer.

2. A method as in claim 1 wherein said inorganic or organic salt is food-compatible or suitable for biomedical applications.

3. A preparation of chitosan which overcomes the drawback of the prior art.

4. The preparation of claim 3, wherein said preparation is selected from the group consisting of :

a) a preparation substantially free of chitosanase;

b) a preparation substantially free of undesirable salts;

c) a preparation substantially free of excess acid; and

d) a preparation which is food compatible and suitable for biomedical applications.

ABSTRACT OF THE DISCLOSURE

Composition of precipitated chitosan polymer containing mixture of certain chaotropic salts as well as methods for making and using same are disclosed. Chitosan polymer preparations produced by such methods are substantially free of chitosanase, undesirable salts and excess acid and retain their physiological as well as biological and physico-chemical properties. The chitosan preparations of the present invention are valuable for the dispensing of biologically active chitosan in forms of drugs or food supplement. These preparations easily dissolve in an aqueous acidic milieu such as the one of the stomach.